Table 3.3.1 Summary of Teratogenic Effects of RadioFrequency Radiation

Exposure conditions	Effect	Reference
64 MHz MRI clinical device operated at power level that produces <0.2°C temperature rise in humans 36 min on day 7 of gestation	Mice C57BL/6J: Eye malformations, mainly microphthalmia Strain genetically predisposed No change in maternal core temperature	Tyndall & Sulick 1991
433 and 906 MHz	Mice: Retarded development *in utero* Reduced weight of thymus at birth No significant change in maternal rectal temperature measured	Saito et al 1986, 1987
2.45 GHz(cw) 5 W/m^2 SAR = 4 W/kg continuous exposure from day 1 to 12 of incubation	Japanese quail: Retarded development in cerebellar cortex Low brain and body weights No difference after 8 weeks of age	Inouye et al 1982
428 MHz 5.5 mW/cm^2 SAR to 47 mW/kg throughout 20 days incubation period	Chick: Delayed embryonic development Embryo-lethal in 60% population Non thermal effect	Saito et al 1991
428 MHz SAR = 3 - 47 mW/kg continuous exposure for 48 h	Chick: Retarded embryonic development	Saito & Suzuki 1993

Other non-thermal effects

In a study on the potential teratogenic effects to clinical levels of magnetic resonance imaging (MRI) a statistically significant increase in the incidence of eye malformations was observed in offspring of mice (Tyndall & Sulik 1991) exposed for 36 min on day 7 of gestation.

MRI includes a strong magnetic field and an RF component of 64 MHz. Although no dosage information was provided, it was stated that MRI devices are limited in the USA to power density levels that do not produce a temperature rise greater than 0.2°C. Eye malformations, primarily microphthalmia, were produced in a C57BL/6J strain of mice that is genetically predisposed to this abnormality (10% spontaneous incidence). The authors claim this to be the first report of a teratogenic effect of MRI. Their measurements of maternal core temperature show bulk heating is not the cause, however, they cannot exclude the possibility of localised heating. While this finding is of interest for medical safety, its usefulness to the scope of the current report is limited due to the absence of dosimetic information. Interpretation is complicated by the high magnetic field strengths and possibility of localised RF absorption.

3.3.2 Implications

Hyperthermia is a known teratogen. Radiofrequency heating is potentially more hazardous than whole body environmental heating. Conventional sources of heating warm the surface of the body allowing the thermoregulatory system to control core temperature via heat receptors in the skin. The slow heating can trigger the synthesis of specific heat-shock proteins that can afford thermotolerance to dividing cells (Walsh et al 1985). However, rapid heating of deeper tissues resulting from absorption of RF energy can cause significant heating and short-circuit the thermoregulatory and thermoprotective mechanisms. The thermoregulatory response to RF heating may be delayed until the temperature of the blood entering the hypothalamus increases. As fetal thermoregulation relies on the maternal homeostatic mechanism the delay would be extended until the maternal blood temperature increases significantly. The human fetus is situated approximately 2 cm below the abdominal skin throughout pregnancy. It is conceivable that localised hot-spots could occur in the fetal brain without initiating a measurable physiological response in the mother.

A study by Lary et al (1982) drew attention to the potential adverse effects from exposure to high intensity RF fields from diathermy medical equipment. A review of teratogenic effects of RF radiation (Lary & Conover 1987) described a number of adverse effects in humans following inadvertent exposure to 27.12 MHz diathermy medical equipment.

3.3.3 Recommendations

The suggestion of risks of harm to the unborn child from any environmental component in modern life is taken seriously by the general population. The potential risk of interference with embryonic and fetal development therefore urgently requires directed research. Sporadic negative reports under different experimental conditions do not quell the concern raised by a single report of an adverse effect. Worst-case models can be established which allow temperature measurement and evaluation of biochemical and developmental effects using *in vitro* embryo culture procedures.

If laboratory animals are used then appropriate adjustments on scale and allowance for resonant frequencies must be determined to allow extrapolation to human exposures. Studies need to be undertaken that test for the possibility of impaired CNS function in offspring that appear normal. It is not sufficient to merely consider gross endpoints such as physical abnormalities. Through links with other common agents such as caffeine, cigarettes, alcohol and drugs, the public appreciates the risk to mental performance. It is most urgent that a substantial data base is established, particularly at intended frequencies and exposure conditions for wireless PCNs.

Sensitive assays such as alteration of neural protein production, synthesis of heat shock proteins and transcription of messenger RNA need to be employed to characterise embryonic stress. These studies should be designed to support research on RF interaction with the developing central nervous system. The potential synergistic effects of environment pollutants and pharmacological agents need to be explored.

3.4 THERMAL/PHYSIOLOGICAL EFFECTS OF EMR

SUMMARY

Heating is the most widely accepted physical mechanism responsible for initiating biological responses and/or changes (whether biochemical, functional or behavioural). A number of repeatable effects including alteration of the plasma concentration of corticosteroids following stimulation of the hypothalamus, teratological effects and death have been produced at SAR levels sufficient to cause significant heating.

Threshold exposure levels for thermal effects in various animal species have been used as the basis for standards for safe human exposure limits (IEEE 1992; ANSI 1992; INIRC 1991; NCRP 1986). However, the relevance of basing exposure standards entirely on thermal effects in laboratory animals exposed acutely to RF radiation is questionable, particularly when chronic exposures to low levels of RF radiation are more appropriate studies for implications to human health. The current data base for non-thermal bioeffects is inconsistent and may not be considered sufficiently robust for such an assessment of dosage.

The issue of cumulative dose from both repeated exposures and radiation from multiple sources has not been specifically addressed.

INTRODUCTION

The best understood mechanism of interaction with electromagnetic RF radiation is that of tissue heating. RF energy interacts primarily with water molecules. Water molecules are dipolar because each one is electrically unbalanced with more negative charge at one end and more positive charge at the opposite end. Thus the molecules align themselves with an externally applied electric field. As RF fields are not static, the water molecules experience an oscillating electric field that changes at the rate of the applied RF frequency. The consequent vigorous agitation of water molecules generates heat. Similar vibrational forces can occur with ions in electromagnetic fields. A detailed explanation of the interaction between RF radiation and molecular components of biological systems is given in a report by the NCRP (1981).

One would expect that alterations in cellular function would, at least, approach a linear relationship of; power: absorbed dose: temperature increase: biological response. In complex biological systems in the animal body the presence of energy deposition hot spots and regional anatomical differences in heat dissipating capability (vascular perfusion, conduction) will distort the curve. Nevertheless, the expectation would be of an evident dose-response. When the biological response is triggered by some other factor (e.g. cell receptor response to ligand or agonist, or depolarisation of cell membrane potential) one might expect an all-or-none response.

3.4.1 Experimental Evidence

No single expression of dosage is perfect for all occasions. There can be difficulties in using an estimate of absorbed dose (SAR) to quantify thresholds of biological effects amongst different animal species with different body mass. This is particularly so for thermally-induced effects, as Gordon (1988; Gordon & Ferguson 1984) has shown a wide variation in temperature elevation for a given SAR for laboratory animals. The resulting biological effect is dependent on both the extent of temperature elevation above the physiological, basal, temperature and the duration for which the hyperthermic insult is maintained. If there is such wide variation for robust effects of whole-body heating it is hardly surprising that apparent discrepancies exist where more subtle non-thermal effects are involved.

It is appropriate, therefore, that physiological responses are used as indicators of interaction with low level EMR. It is known that in response to non-specific stress, the hypothalamus stimulates the adrenal complex to secrete the glucocorticoids, corticosterone and cortisol. This response has been used by some researchers to attempt to quantify microwave-induced heat stress in laboratory animals.

There is remarkable consistency in the correlation of SAR and rectal temperature in data reported by the same author (Lotz & Michaelson 1978; Lotz & Podgorski 1982; Lotz 1983) from rats (2.45 GHz) to monkeys (1.29 GHz) where SAR around 3 W/kg induced approximately 1°C temperature increase (Table 3.4.1). This is not consistent with reports on correlating thermal physiology with body mass (Gordon 1987; Gordon & Ferguson 1984). Meanwhile, exposure at 918 MHz to SAR of 4 W/kg for 10 h produced no change in rectal temperature in rats (Moe et al 1976). Repeating the exposure over 21 days did not alter the levels of plasma

Table 3.4.1 Some Thermal and Physiological Responses in animals exposed to microwave radiation

Exposure conditions	Effect	Reference
2.45 GHz (cw) 500 W/m^2 SAR = 8-10 W/kg 0.5-1 h 200 W/m^2 SAR= 3.2 W/kg 2 h	Rats: Significant increase in plasma corticosterone levels Rectal temperature increased 0.7-1.5°C	Lotz & Michaelson 1978
2.45 GHz 100 W/m^2 SAR = 2.5 W/kg 16 h	Rats: No change in plasma corticosterone levels No increase in rectal temperature	Parker 1973
918 MHz 100 W/m^2 SAR =4 W/kg 10 h per day x 21 days	Rats: No change in plasma No change in rectal temperature	Moe et al 1976
918 MHz 25 W/m^2 SAR = 1W/kg 10 h per day x 91 days	Rats: No change in serum cortiscosterone Rectal temperature unchanged	Lovely et al 1977
1.29 GHz 380 W/m^2 SAR = 3-4 W/kg 4-8 h	Monkeys: Significant increase in plasma cortisol Effect can be masked by diurnal variation in hormone levels Rectal temperature increased by 0.9-1.6°C	Lotz & Podgorski 1982 Lotz 1983
35 GHz SAR 12 w/kg 15 min	Rats Circulatory collapse and death No significant increase in rectal temperature	Frei 1994

corticosterone. These results apply to whole body exposures but show that physiological response to stress may provide a useful biological endpoint.

3.4.2 *Behavioural Effects*

There have been a number of publications on the alteration in behavioural patterns in mammalian species exposed to microwave radiation. Traditionally, rats are used as subjects that can be trained to perform functions or to learn a route to food through a maze. Conditioned response behaviour typically involves a food morsel as a reward for pressing a lever. Short-term exposures to microwave radiation can alter trained performance, motor behaviour and thermoregulation. Interruption of this behaviour pattern requires a significant stimulus. The thermally-induced reaction to whole-body microwave exposure is recognised and forms the basis of the development of the ANSI safety standard. Comment on its suitability is made in a separate chapter on standards and regulations. The subject has been extensively reviewed and the current text will only deal with recent significant additions to the literature on the effect of pulsed microwaves.

Auditory perception by humans of pulsed microwaves requires small amounts of total absorbed energy (approx. 2 to 40 μ J/cm^2 energy density at 2450 MHz, according to Chou et al 1982). Yet this pressure-wave-induced sensory response has not been reported to affect behaviour patterns in non-human primates. This factor indicates the relative severity (and insensitivity as an experimental endpoint) of microwave-induced-hyperthermia-mediated behavioural responses.

A recent study (D'Andrea et al 1994) used only four male rhesus monkeys exposed to pulsed microwaves (5.62 GHz, 100 pps, 2.8 μ s pulse duration) for 20 mins at high peak powers where the SARs ranged from 2 to 6 W/kg. Significant alterations in lever responding and reaction time were observed during exposures at 4 W/kg, but not at 2 W/kg. This result has remarkable similarity to that reported earlier by de Lorge (1984). The energy densities within the pulses ranged from 156 to 776 μ J/cm^2. In a review article by D'Andrea and de Lorge (1990) it was stated that "at 918 MHz it is reasonable to assume that the threshold for behavioural effects lies within the range 0.9 - 2.0 W/kg." Some of these studies were carried out at 918 MHz frequency and found a threshold SAR value around 2 W/kg for disruption of behavioural activies in male rats (Moe et al 1976; D'Andrea et al 1980; Lovely et al 1977, 1983). Effects observed included reduced food intake, decreased blood sugar level and some level of increased activity. The exposures were repeated daily for many weeks. Altered behaviour was reported in studies carried out at 2450 MHz frequency (cw) at SAR values from 0.14 W/kg (D'Andrea et al 1986; De Witt et al 1987) to 3.2 W/kg (Lovely et al 1983). In a review of the topic D'Andrea and de Lorge (1990) specify that the SAR threshold for significant behavioural effects from long-term exposure at 2450 MHz is between 0.4 - 0.7 W/kg, and at 915 MHz is between 0.9 - 2.0 W/kg. By comparison, short-term acute exposure behavioural changes were associated with a minimum whole-body temperature increase of at least 1°C from SARs approximately 4 W/kg.

A rather more interesting finding that seems to have escaped much publicity is the depressing effect of microwave exposure on memory function in laboratory rats (Lai et al 1989, 1994). The radial-arm maze test was used to demonstrate impaired short-term memory function following an acute exposure of 45 min to 2.45 GHz RF at power density of 1 mW/cm^2 and estimated whole body SAR 0.6 W/kg. The mechanism of effect has been proposed as one of activation of endogenous opiods in the brain resulting in decreased cholinergic activity in the hippocampus (learning centre). In addition, DNA in brain cells was reported to be damaged, assayed by electrophoretic techniques (Lai, private communication). The effects were observed with both pulsed (2μs, 500 pps) and c.w. waveforms. Breakage of DNA in the CNS and testes has also been reported recently (Sarka et al 1994) using the same sensitive electrophoretic technique, following exposure to microwaves at 1.18 W/kg SAR.

An interesting finding in irradiated laboratory rats that is difficult to explain, in terms of a mechanism reported circulatory collapse and pools of blood in the gut (similar to that occurring in heat stroke) followed by death. The effects have been observed at microwave frequencies of 1, 10 and 35 GHz (Frei 1994) at SAR 12 - 14 W/kg. Death occurred within 15 to 35 min. For exposures at 10 and 35 GHz no significant change in rectal temperature occurred, as penetration at this frequency was limited to the skin.

4.0 THE CELL MEMBRANE, ION EXCHANGE AND CELLULAR EFFECTS OF EMR

SUMMARY

The cell membrane is considered as the primary site for EMR interaction with cellular systems. Interference with membrane-mediated signal detection, transduction, or amplification processes may underlie many of the biological non-thermal effects reported in the literature. The mobilization of cellular calcium ion (Ca^{2+}) by electromagnetic radiation, or other stimuli, is an important biological response in the regulation of cellular activities.

A common finding is that calcium ion concentrations and Ca-dependent cellular processes are affected by EM fields. While the data on calcium efflux is equivocal, it cannot be ignored that independent research groups have reported an increase in $^{45}Ca^{2+}$ efflux from brain tissue exposed to low levels of microwave or RF radiation, usually modulated around 16 Hz. The positive effects were supported by a further report of altered calcium ion efflux in human neuroblastoma cells exposed to 915 MHz modulated at 16 Hz. As the effective SAR in all of these studies was below 0.05 W/kg this suggests that a non-thermal mechanism of interaction exists that depends on the extremely low frequency modulation component. The efflux assay system requires further research which may yield useful information in determining the means by which EMR exposure conditions can sensitise and affect cell membrane responses. The significance for human health of such transient ionic changes is uncertain.

Apart from gross effects on metabolism and membrane structure that may result from substantial bulk heating, there is good supporting evidence of discrete changes in cell membrane permeability where heating does not occur. There is gathering evidence of non-thermal effects of EMR. Molecular lipid composition of bilaminar membranes is altered at specific structural phase-transitional temperatures. Evidence is given of enhanced permeability of lymphocytes to sodium at a specific temperature rather than due to a temperature increase (Liburdy 1992). Membrane stability is reduced by a downward shift in the lipid phase-transition temperature. Microwave radiation (unmodulated) has been shown to reduce the phase-transition temperature. Calcium ions are implicated in providing structural integrity by cationic bridges in the cell membrane.

It is unlikely that any single interactive mechanism is responsible for the range of effects observed at the cellular level. Research is needed at the level of ionic

channels to demonstrate the mechanism of ion transport into and out of cells during exposure to EMR.

4.1 Introduction

RF radiation at frequencies up to several hundred GHz are known to be nonionizing forms of energy, because their quantum energy is too low to cause physicochemical or biological effects by ionization of molecules. Ionizing radiation readily produces chromosomal damage in cells. This is the major reason why biologists and physicists have long held the view that non-ionizing electromagnetic energy can produce detectable effects only via mechanisms involving significant heating in cells, such as the non-excitable cells of the immune system. During the past 10 years, however, bioelectromagnetics research has shown that nonionizing electromagnetic energy can induce a variety of biological effects not only by thermal interactions but also through interaction mechanisms that do not involve any macroscopic heating. Low-intensity field effects, which apparently are not induced by thermal interactions, are referred to in the literature as athermal, or alternatively, non-thermal field effects.

4.1.1 *Cell Membrane*

Cell membranes have a high content of fat molecules (phospholipids) that partition each cell from its neighbour. This plasma membrane is comprised of a double layer of phospholipid molecules, approximately 40 Å thick. A steady state potential of approximately 0.1V (equivalent to an electrical gradient 100kV/cm) exists across the membrane of most cells. Glycoprotein molecules protrude through the cell membrane and form a strongly negatively charged glycocalyx on its outer surface which provides a receptor site for hormones, antibodies, neurotransmitter molecules and cancer promoters. Tissues comprise of aggregate of cells separated by narrow fluid channels, approximately 150 Å wide, through which these substances travel to reach binding sites on cell membrane receptors (Adey 1992). This intracellular space provides preferred strongly conducting pathways for electromagnetic fields, having considerably lower electrical impedance than cell membranes. Cell to cell communication occurs via this route.

Within cells, molecular systems mediate essential processes of metabolism, reproduction and responses to environmental stimuli. Cells interact primarily with their physical and chemical environment and communicate through the cell membrane. The enveloping cell membrane acts as both sensor and effector.

As a sensor, it detects altered chemistry in the surrounding fluid. It offers a path for inward signals generated on its surface by a range of stimulating ions and molecules, including hormones, antibodies and neurotransmitters. As effectors, cell membranes may secrete substances synthesized internally, including hormones, antibodies and structural proteins such as collagen. Both the sensor and effector functions are susceptible to manipulation by natural or imposed electromagnetic fields. The interaction triggers a cascade of events at the biomolecular level that may profoundly alter cell growth activity and proliferation. It is suggested that receptor-mediated influx of calcium in epidermal cells or mast cells is due to molecular vibration at the receptor sites and is not voltage-activated. Such an influx is observed, for example, following binding of the epidermal growth factor (EGF) to its specific receptor resulting in a 4-fold increase in intracellular calcium while the membrane potential was unchanged (Moolenaar et al 1986).

4.1.2 *Ion Channels*

Present in the cell membrane are specialised channels for ion transport that regulate ion fluxes required to regulate proper cell function. Important examples of such signal transduction events at the cell membrane are the binding of extra-cellular ligands (e.g. hormones, proteins) to cell surface receptor sites, and ion-channel transport across the lipid bilayer. Both events result in structural changes in the bilayer organisation that initiate the activation of diverse biochemical pathways transducing signals to internal cellular sites (Gardner 1989). Calcium plays a key role in this signalling process.

Such an effect is among the earliest detectable events triggered by binding of a ligand (e.g. antigen, receptor antibody or mitogenic lectin) to an appropriate receptor on the outer cell surface. The subsequent cascade of cellular reactions in lymphoid cells is best understood for T- cells (Weiss & Imboden 1987). Ligand induced Ca^{2+} mobilization is reflected by an initial rise in the cell's internal concentration of calcium ions $\{Ca^{2+}\}_i$ which is caused by inositol 1,4,5-triphosphate-induced release of Ca^{2+} from intracellular stores and followed by a sustained receptor-mediated Ca^{2+} influx from the extracellular medium. Perturbation of these events with chemical agents (such as Ca^{2+} channel blockers, Ca^{2+} - specific ionophores) or lowering the extracellular Ca^{2+} concentration by using chelators can alter Ca^{2+} membrane fluxes and subsequently modify cellular activity. Effects produced include altered cell proliferation, secretion, motility, or cytotoxicity (Harris et al 1988; Lichtman et al 1983). Ca^{2+} regulation in lymphoid cells of the immune system could be similarly affected by appropriate EMR interaction.

The interaction of EMR fields depends on the efficiency of energy transfer to components in the cell membranes. Dipolar components, such as polar amino-acid side chains and cell surface bound water molecules will undergo rotational field orientation at microwave frequencies. At sufficiently high levels of specific absorption rates (SAR > 1-10 mW/gm) this motion will result in heating (ANSI 1982). Localised heating at the cell membrane can alter the phase of phospholipid molecules. The phase transition temperature may be altered under such conditions.

4.2 *Experimental Evidence*

According to Cleary (1990) there is definite evidence, from *in vitro* studies, of direct, frequency-dependent and field-strength dependent alterations of various types of mammalian cells by RF radiation. The variety of effects suggests multiple macromolecular mechanisms. There is some evidence to suggest that EMF-altered Ca^{2+} regulation is an early trigger of field effects in cells of the immune system. Given the established role of Ca^{2+} in the regulation of lymphocyte proliferation it has been proposed that EMR-altered Ca^{2+} regulation can modify lymphocyte DNA synthesis (Walleczek 1992). Most of this research has been carried out at ELF where, for mitogen-activated cells, EMF signals were effective modulators of both Ca^{2+} uptake and DNA synthesis. These results suggest that activation of transmembrane Ca^{2+} signaling is required in order to obtain field effects on both Ca^{2+} uptake and DNA synthesis. Similarly, increase in calcium uptake and DNA synthesis has been reported following single exposures at microwave frequencies (Cleary 1990). The use of an agonist co-promoter further reduced the exposure threshold level.

Evidence is given of enhanced permeability of lymphocytes to sodium at a specific temperature rather than due to a temperature increase (Liburdy 1992). Exposure for 90 min to 2450 MHz at 6 mW/gm power density produced no effect at 40°C, but resulted in a two-fold increase in accumulation of $^{22}Na^{+}$ in rat lymphocytes at 37°C. This cell type is critical to the immune system and is reported to exhibit a loss of cell surface proteins and alteration of membrane permeability when exposed to microwaves at normal body temperature. Inhibition of the intracellular Na^{+}/K^{+} pump (and consequent accumulation of Na^{+} ions) has also been reported in human erythrocytes exposed to 2450 MHz frequency at 37°C (Allis & Sinha-Robinson 1987).

The findings that microwave fields influence both passive and active sodium transport in eukaryotic lymphocytes and erythrocytes at 37°C may have important implications for the immune system. Na^+/K^+ transport is critically involved in intracellular enzyme function, and regulation of cellular growth and functions. The transport of sodium, potassium and calcium is vital in lymphocyte proliferation and maturation, and antibody production. It is possible that microwave-induced alterations of cation transport can perturb nuclear processes such as DNA synthesis. It is noteworthy that increased DNA synthesis has been reported in human lymphocytes following a single exposure for 2 h to 2450 MHz, at SAR of 5 to 50 W/kg at 37°C (Cleary et al 1990 a). This laboratory has also reported increased proliferation and transcription of glioma (human brain tumour) cells using similar exposure conditions (Cleary et al 1990 b). The altered rate of growth was maintained for up to five days after the irradiation.

4.2.1 *Ion Fluxes*

In many types of cells (neuronal, cardiac, secretory) fluctuations occur in membrane potential and are accompanied by oscillations in the concentration of intercellular ions. The most commonly studied effect is that of altered concentration of intracellular free ionized calcium $[Ca^{2+}]_i$ resulting from Ca^{2+} influx via voltage-sensitive calcium channels in the cell membrane. So called oscillatory Ca^{2+} responses also occur in non-excitable cells (Fewtrell 1993) in response to a stimulus at the cell membrane receptors. Early experiments reported alteration of Ca^{2+} efflux from avian brain tissue irradiated with RF modulated at 16 Hz (Bawin et al 1975; Adey 1981; Blackman et al 1982).

The use of sensitive endpoints for bioeffects research seems to be inevitably accompanied by publication of contradictory findings. The very nature of highly sensitive systems involving fluxes in ionic composition suggests that a response may be elicited by some aspect of experimental conditions that might differ between separate laboratories testing the same biological endpoint. The issue of calcium efflux response to RF fields is no exception. Early studies exposed isolated chick brain tissue to power densities 10-20 W/m² at 147 MHz and reported statistically significant increases in labelled calcium ion ($^{45}Ca^{2+}$) efflux when the field was modulated at frequencies from 6-20 Hz. The SAR was estimated as 0.002 W/kg and the effect considered to be non-thermal with the maximum effect at 16 Hz modulation. No effect was observed from unmodulated fields (Bawin et al, 1975). This work was replicated in other laboratories at 450 MHz carrier wave frequencies where the effect was observed at specific modulation frequencies and at specific power density windows (Sheppard et al 1979). Enhanced efflux of calcium ion from chick brain tissue was also reported for power density windows at a frequency of 147 MHz modulated at 16 Hz, but was subsequently found to be related to the temperature of the preparation (WHO 1993; Blackman et al 1991).

Calcium ion efflux was increased in rat synaptosomal preparation by exposure to 450 MHz, amplitude modulated at 16 Hz with a power density of 10 W/m^2 (Lin-Liu & Adey 1982). Meanwhile, a number of negative results were reported in rat brain tissue preparations exposed *in vitro* to 1-2 GHz, pulse modulated at 16 Hz and other ELF frequencies with power densities of 10 to 150 W/m^2 (Shelton & Merritt 1981; Merritt et al 1982). However, the negative studies were not exact replications of the exposure conditions and experimental protocol used by Bawin or Blackman.

Positive effects have been reported at carrier frequency relevant to cellular telephones when human neuroblastoma cells in culture were exposed to 915 MHz at SAR of 0.005 and 0.05 W/kg modulated around 16 and 60 Hz (Dutta et al 1984, 1989).

Detection of changes in calcium concentration can be confounded by complicated dynamics of calcium cytoplasmic concentration and intracellular stores of free Ca^{2+}. The location of these stores varies with different types of cells. Muscle cells are known to store calcium in a specialised organelle, the sarcoplasmic reticulum, however, it is not understood how Ca^{2+} ions are transported across its membrane into the cytoplasm. The structure and intracellular location of Ca^{2+} stores in other cells is still a subject of debate (Rossier et al 1991; Krause 1991). It is known that intracellular Ca^{2+} concentration changes as calcium is released from stores or as Ca^{2+} traverse the cell plasma membrane in response to stimuli. Excitable cells are thought to respond when depolarisation of the cell membrane potential activates voltage-sensitive Ca^{2+} channels and allows influx of Ca^{2+}. Non-excitable cells are thought to lack voltage-sensitive Ca^{2+} channels, nevertheless variations in extra cellular Ca^{2+} concentration modulates the frequency Ca^{2+} oscillations in many cells (Kawanishi et al 1989).

Despite the difficulties in interpretation of reported results, the fact exists that the literature contains a number of reports of imbalance of ionic concentration resulting from exposure to EMR. A recent attempt to verify a report of enhanced calcium efflux (Schwartz et al 1990) in amphibian cardiac muscle was unsuccessful (Wood et al 1993) and demonstrated the wide variability in intracellular calcium levels. The excised hearts were irradiated with RF 240 MHz amplitude modulated by 16 Hz at SARs up to 0.36 W/kg. The studies are important as calcium plays a critical role in the physiology and contractile operation of cardiac muscle. It is important that these studies are repeated with more sensitive ionic detection procedures. A study is currently being developed to use computer acquired data from the confocal microscope and fluorescent molecules to obtain information in real time on Ca^{2+} movements.

4.3 Possible Mechanism

It is thought that Ca ions form cationic bridges with protein/phospholipid moieties on the cell surface. When the Ca ions bind to the polar, anionic head groups of phospholipids in the membrane they provide stability to the membrane by raising its structural phase transition temperature (to 40°C). Exposure to microwave fields reduces the phase transition temperature to around 37°C resulting in destabilisation of the bridges and release of protein from the cell surface (Liburdy 1992). At the same time membrane permeability is altered. This effect of protein shedding in lymphocytes and erythrocytes has been recently reported after brief exposure of ≤ 30 min at 2450 MHz (cw) and 60 mW/kg (Liburdy 1992, 1994).

It is thought that loosely bound proteins play an important role in the transduction of signals to integral proteins that span the bilipid membrane. Exposure of liposomes to 2450 MHz at only 0.6 mW/kg for 5 min resulted in a reduction in the main structural phase transition temperature from 39.5 to 38°C (Liburdy 1994). Radiofrequency radiation fields have also caused the release of immuno-globulin (Ig) from antibody receptor sites on the surface of B-lymphocytes (Liburdy & Wyant 1984) at non-thermal exposure conditions (2450 MHz, SAR = 0.117 mW/gm).

Free Radicals

Due to its extreme reactivity towards macromolecules, hydroxl radical (OH^{*}) is a highly toxic moiety that is probably implicated in many molecular biological effects. It is known to cause strand breakage and base modification in DNA, crosslinking of nucleic acids and proteins, enzyme inactivation and lipid peroxidation. Thus, it has the capability to significantly intefere with and alter the growth and development processes of cells. Exposure to many chamical compounds and non-ionizing radiation are capable of inducing free radical production at the cellular level.

There is increasing support for the theory that free radicals play an important role in discrete, important sub-cellular events during exposure to microwaves. The field of magneto-chemistry is beginning to have an impact on the understanding of subtle effects in molecular biology of cell systems. Chemical bonds consist of paired electrons with opposite spins. Free radicals are highly charged and can only form bonds between radicals of opposite spins. Electron spins may be altered by EM fields and radicals prevented from uniting. Recent information on the small unstable molecule, nitric oxide (NO), as a physiological mediator has shown the importance of oxygen free radicals in biological systems. NO is understood to modulate neurotransmission and regulate cerebral arterial blood flow and has been implicated in the pathogenesic of Alzheimer's disease.

The microwave-induced lowering of phase transition temperature and increasing membrane permeability is inhibited by the presence of antioxidants, thereby implicating free radical involvement (Liburdy 1993). A number of laboratories have reported enhanced permeability to sodium cation in erythrocytes during exposure to microwave fields (Liburdy & Penn 1984; Clearly et al 1982; Allis & Sinha-Robinson 1987; Lotz & Saxton 1989; Liburdy 1992).

4.4 Implications

Studies on alterations in calcium flow generally measure the end result of an interaction, and mostly speculate on the actual mechanism of flow and its initiation process. Studies with RF and microwave exposures tend to present phenomenological data. The process of trying to correlate between studies that often use different exposure protocols, different cell types and subsequent variation in dosimetry is complicated further by the absence of an accepted mechanism by which the effect occurs.

Much of the literature on cellular effects of EMR lacks a dose-response and reported effects are considered to be due to some non-thermal mechanism. Biochemical pathways in signal transduction are becoming better understood, but much of these so-called non-thermal mechanisms are speculative. There are serious gaps in the knowledge which need to be studied rather than dismissed as fanciful ideas. Studies that are directed towards understanding the underlying principles in cellular non-thermal reactions need urgent support, particularly where there are genuine health implications, such as in the control and development of neural proteins, or alteration in the cell cycle kinetics in brain glioma cells. Some interesting data has been published on the effects of microwave radiation (27 and 2450 MHz, single exposure for 2 h), on glioma cell proliferation, assayed by ^{3}H-thymidine incorporation in DNA (Cleary 1990). Similar accelerated growth effects were demonstrated for human lymphocytes exposed to SAR of 5 W/kg. The altered growth rates lasted for up to 5 days after the irradiation.

There is a flaw in the notion that applies "safety" standards that only refer to measurable gross physical quantities. When considering biological consequences it is essential to understand that the extreme lethal levels can be less harmful to the organism/individual than a seemingly negligible change that modifies the behaviour and development of cells. For example, it is now well understood that during early pregnancy, at the time of closure of the mammalian neural tube, hyperthermia can have devastating effects for the developing embryo and fetus. Large temperature increase results in embryonic death and abortion. However, modest temperature increase can interfere with the critical developmental processes and result in a range of severe abnormalities of the central nervous system including exencephaly, micrencephaly, microphthalmia (Edwards 1993).

While some opinions might consider that apparently esoteric studies on a specific cell reaction in a petri dish has little relevance to the "real world" of human health hazards from irradiation with EMR, the advantages of these studies cannot be overlooked. Analysis of molecular and ionic behaviour in EM fields is fundamental to understanding whether EMR can perturb enzymatic and biochemical control pathways and interfere with cell growth and development. Of course, reactions in a simplified controlled *in vitro* environment cannot be directly extrapolated to the *in vivo* situation where homeostatic humoral and thermal feedback control systems dominate. *In vitro* studies are far more sensitive than animal studies and allow more precise quantification of dosage and control of environmental variables. Any safety recommendation or guideline needs to take account of the possibility of risk from all mechanisms. The current ANSI standard apparently only considers data based on animal behavioural changes resulting from a gross thermal effect that elevates the whole body temperature by at least 1°C. Meanwhile, there are many reports of biological effects that cannot be attributed to heating and are, consequently, ignored by the Safety Standard. The reported biological effects are clearly responses in some, as yet, undefined way to the EM field.

At the other end of the spectrum human epidemiology studies are extremely crude, by comparison. There are enormous difficulties in gaining high statistical power due to the wide range of environmental factors and complicated behavioural patterns of mobile populations. It would need to be an extremely robust effect that could be detected above the background "noise" level. Many environmental factors, chemical, UV, power lines, stress, airborne pollutants, are said to be associated with human cancers and will mask the effects of another potential agent such as EMR. Without knowledge gained from laboratory research such surveys would not have a realistic hypothesis to test and, therefore, would have little chance of uncovering useful information. It is rather optimistic to propose that a population survey will provide scientifically acceptable information when no known mechanism exists and a probable outcome cannot be predicted.

5.0 CANCER STUDIES

SUMMARY

There is increasing concern about the possibility that RF exposure, particularly from cellular telephones, may play a role in the causation or promotion of cancer, particularly in blood forming areas of the CNS. Experimental evidence from *in vitro* and *in vivo* studies indicates that EMR exposure does not produce adverse genetic effects and is, therefore, unlikely to have a direct effect on tumour initiation. There is, however, some evidence of subtle changes in cell behaviour and proliferation rate that could be consistent with an effect at the level of tumour promotion. *In vivo* studies have shown accelerated rates of growth of certain tumours when microwave exposure is applied after the initiation of known carcinogens. Stress is an important potential confounder. Dosimetry and the effects of resonant frequency, body orientation are important factors that must be carefully controlled. Cellular studies give evidence of microwave-induced neoplastic transformation. There is no convincing evidence that athermal microwave irradiation produces clastogenic effects.

The potential for direct effects on DNA through enhanced absorption of microwave frequencies has not been supported by subsequent studies. Results of studies related to cancer induction by microwave or RF radiation are equivocal. Many early studies are severely limited by inadequate dosimetry and poor histopathology.

Further research in this area is needed to resolve many of the controversial issues. A number of long-term exposure studies are about to begin (three are in-progress). It will be interesting to see if there is any similarity in the design that will allow effective cross-comparisons of data.

5.1 Molecular Mechanisms in Radiation Induced Cancer

The relative binding energies of EMR and the organic molecular bonds in mammalian cells provide the limitation on the likelihood of cancer production by direct action on DNA. The binding energy of typical chemical organic molecules is approximately 300 kJ per molecule, i.e. 3 eV or 10^{-19} J per single bond (Burkart 1993). Whereas ionizing radiation particles release large energies (approx. 5 MeV, or 10^{-13} J) and easily damage DNA or cellular components, the relatively low energy levels in EMR fields are generally considered to be incapable of damaging DNA directly. The photon energy is about 10^{-3} eV at 300 GHz and decreases linearly with decreasing frequency (NRPB 1993).

However, biological response to absorbed radiation energy is complex and depends on many parameters. A common by-product of radiation interaction with water molecules is the formation of chemically-reactive free radicals. With low level ionizing radiation, the activity of free radicals is thought to constitute the major cause of cancer production while direct action on the nuclear target molecules occurs with high level exposures. Radicals can react with DNA of the cell nucleus and damage the genetic code.

The hormone melatonin, has been suggested as an inhibitor of cancer by way of its ability to act as a potent free radical scavenger. Suppression of melatonin production is associated with increased breast cancers in women. Evidence is accumulating to show that such free radicals are formed in cellular responses to EMR (Liburdy). There are differences in biological sensitivity due to the type of cell and its position in the cell cycle. Cellular kinetics of tissues are important in their response to radiation. Generally, cells in S-phase or M-phase would be expected to be most sensitive to physical insult. The environmental conditions, temperature, degree of hypoxia or presence of antioxidants are important in the intracellular interactions.

While it is generally accepted that incorrectly repaired or unrepaired modifications of the DNA molecule are the main cause of radiation induced cancer, other "epigenetic" mechanisms have also been recently proposed. The epigenetic effects include; initiation of membrane lipid peroxidation, or loss of intercellular signalling such as gap junction mediated transfer of messenger molecules (Lowenstein 1979). Subtle changes in the genome that do not adversely affect the proliferative capabilities but may impair regulation of cell growth can lead to the late somatic effects of cancer. Loss or alteration of crucial genetic information in gonadal cells may create a risk of congenital disease.

Malignant cells are defined by certain characteristic features such as unrestrained proliferation, angiogenesis (the ability to attract blood supply), infiltration into neighbouring and distant tissues, and the evasion of attacks by the immune system. Such characteristics could be the result of genetic modifications, i.e. a somatic mutation, or result from epigenetic changes. Epigenetic factors, which do not lead to irreversible changes in the primary structure of the genetic code, could produce de-differentiation, activation and expression of normally suppressed genes involved in the production, binding or signalling of growth factors, inactivation of regulatory genes, or the loss of growth controlling cell-cell-interactions (gap junctions) between a transformed cell and its environment. It may well be that both genetic and epigenetic factors have to act in parallel to let a cell escape the division-restraining signals of its environment (UNSCEAR, 1986).

The origin of cancer is not well understood. However, some quantitative models of cancer induction based on simplified hypotheses have been proposed (Whittemore 1978), based on the concept of multi-stage carcinogenesis, initially developed as a model for skin cancer in mice (Farber 1980). The currently accepted model of carcinogenesis involves a multistage process (NRPB 1993; Cridland 1993) of at least three stages: *initiation*, involving genetic mutation of one or more cells; *promotion*, involving the multiplication and accumulation of damaged cells; *progression* during which further genetic abnormalities accumulate resulting in increased malignancy. In addition, increased proliferation may be associated with carcinogenesis by fixing and amplifying naturally occurring genetic damage, and thus may serve a role as a co-promoter.

It is believed that *initiation* can occur in response to a single brief exposure to an agent, and that it becomes a permanent change occurring within a single mitotic cycle. It probably involves the production of a stable genetic mutation. *Initiation*, is generally considered to occur in the genome. Further steps towards an overt malignancy are generally assumed to be epigenetic in origin, *promotion* and *progression*. An alternative hypothesis can be based on two initiation events, the activation of an oncogene and inactivation of a tumour suppressor gene. *Promotion* is typically a more protracted process requiring repeated exposure to the promoting agent. It is usually reversible if the promoting agent is removed and is therefore unlikely to result from genetic mutation. Promoting agents induce cellular proliferation which allows initiated cells to multiply, expressing an altered phenotype. The best characterised pathway by which promoters can affect cell proliferation involves interference with normal cellular control system through cell surface receptors. Cell growth factors bind to specific receptors which transfer the signal across the plasma membrane and activate signal transduction, biochemical pathways for cell growth and DNA synthesis.

Many cellular phenomena may also be relevant in the development of a transformed cell. At the level of DNA, any change affecting the primary structure, and hence its function, may also affect growth control. Secondary influences, such as endogenous or exogenous growth factors, e.g. female steroid hormone in breast tissue, or levels of melatonin, may increase the probability of initiated cells passing through the stages of promotion and progression to cancer. The administration of exogenous drugs can also affect promotion. Oncogene activation involves the induction or enhanced expression of gene products in growth and differentiation.

5.2 Cellular studies

Cancers result from the multiplication of cells which exhibit abnormal, malignant behaviour and tend to invade and destroy adjacent tissue, or by metastasising they invade other body tissues. Usually, these cells grow at an accelerated rate and often appear to be less differentiated than normal cells.

There have been suggestions that exposure to EM fields may result in an increased risk of cancer. The equivocal nature of such epidemiological reports at ELF has sustained the debate, and recent claims of brain tumours caused by use of cellular telephones have fuelled speculation.

It is important that biological aspects of EMF exposure are fully investigated to determine whether any mechanism exists by which such fields could affect carcinogenesis. Biological tests for identifying potential carcinogens involve either whole animal or cell culture systems which, ideally, should be used in a complementary manner. Each test type has advantages and limitations, with neither being ideal systems. Cellular studies have the advantage that they provide a rapid screening method for potential effects, have greater sensitivity to weak carcinogens, and are more amenable to detailed molecular analysis, thereby allowing study of underlying mechanisms of interaction. Cell transformation assays represent a method for assaying changes consistent with tumorigenesis without knowing the genetic nature of the damage giving rise to the change. After plating at low density, transformed cells, which have an altered phenotype, may be identified using morphological criteria. The problem with existing cell transformation assays is that they are generally based on established cell lines which are known to be atypical. The C3H/10T$^1/_2$ transformation assay has been used to investigate the effects of electric and magnetic fields.

Signal transduction pathways are complicated systems, but some important elements include inositol phosphate metabolism, intracellular calcium ion concentrations, and activation of specific protein kinase enzymes. As these represent important aspects of normal cell growth, detection methods have been developed in assays for study of the cellular effects of EM field exposures as a potential promoting agent, apart from the endpoint of increased proliferation. Tests for elevated enzyme activity such as ornithine decarboxylase, 5[1] - nucleotidase, and ATP-ase are commonly used. DNA synthesis is an essential prerequisite for cell division and is, therefore, one of the most commonly used endpoints of enhanced proliferation.

In the process leading to tumour progression, an initiated cell is acted on by a tumour promoting agent and produces a clone of cells with altered genotype, or genetic complement. During *progression* these cells change from pre-malignant to increasingly malignant phenotype by undergoing loss of growth control and acquisition of invasive behaviour. This may require chromosomal aberrations that inactivate tumour suppressor genes (Cerutti 1988).

Experimental evidence

Initial studies claiming preferential absorption of microwaves by DNA molecules, and therefore giving evidence of direct interaction, have not been confirmed in subsequent studies in different laboratories. It was reported that microwave absorption in purified DNA was greater than for water at 8-12 GHz (Swicord & Davis 1982). Later studies using plasmid DNA (ranging in size from 5 to 30 kb) in aqueous solution found no evidence of enhanced absorption of microwaves over a range of frequencies from 0.1 to 12 GHz (Gabriel et al 1989; Foster et al 1984).

Studies on the potential mutagenic effect of RF radiation have used fungi and yeast and detected no effect at frequencies of 2.45, 8.7175, 9.4, 17, and 70-75 GHz with SAR of 10-30 W/kg.

Determining the rate of DNA synthesis provides a measure of cell proliferation. Before cells can undergo normal mitotic division they must replicate their DNA during the well-defined S-phase stage in the cell cycle. Incorporation of ^{3}H-thymidine is a commonly used assay. Cultures of normal human lymphocytes are widely used.

Transcriptional regulation is a key factor in the control of cellular growth. A number of oncogene products, including those encoded by c-myc, -fos and -jun have been shown to function as transcription factors.

The cell studies that are continually referred to as evidence of potential carcinogenic effects of microwave radiation are those showing enhanced proliferation (Cleary et al 1990 a, b) and cell transformations (Balcer-Kubiczek & Harrison 1985, 1989). Cleary et al reported increased proliferation in human lymphocytes and increased rate of gross transcription in LN71 human glioma cells. However, a recent study, given as an offered presentation at the BEMS conference (Stagg et al 1994) showed the absence of a convincing effect at exposure conditions similar to that emitted by cellular telephones. It is typical of this field of research that similar studies never actually attempt to replicate the protocol used in studies reporting an effect. The work of Cleary et al used frequencies of 27 and 2450 MHz, cw, applied for 2 h and quoted estimated SAR for so-called isothermal exposures at relatively high levels of exposure. They used human glioma and T-lymphocyte cells. The study by Stagg et al used a similar assay for incorporated ^{3}H- tdr and quoted values for power densities up to 3.7 mW/cm^2 RMS values. The RF field was 837 MHz, TDMA pulse modulated and was applied for 24 h to two cell types. Different results were obtained for each cell type. Primary glial cells (rats) showed no effect on DNA synthesis. However, C6 glioma cells showed a significant increase in thymidine incorporation, although curiously there was no significant difference in cell doubling times.

Cell transformation assays test for genetic alteration that gives rise to an altered phenotype that is readily identified by the morphological appearance of the transformed cells. The main problem with this test system is that the cell lines used are generally atypical. The strongest evidence of microwave radiation producing malignant transformation abnormalities comes from the work of Balcer-Kubiczek and Harrison (1985, 1989, 1991). They exposed the mouse embryonal fibroblast line C3H/10T$^{1/2}$, which are commonly used in this assay, and which contain already genetically altered chromosomes. Exposure for 24 h to 2.45 GHz, pulse modulated (120 pps) and SAR 4.4 W/kg, together with X-irradiation, promoted transformation of cells. The effect was enhanced significantly when the chemical promoter TPA was added. Irradiation with microwaves and X-rays produced additive effects, suggesting that they produce effects at different targets, possibly supporting the concept of epigenetic interaction of microwaves.

A recent study (funded by Motorola) has failed to show any effect on proliferation rate of glial cells following 24 h exposure to up to 3.7 mW/cm^2 at 837 MHz for 24 h (Stagg et al 1994). Co-promotion of transformed fibroblasts was not enhanced (Cain et al 1994). However, there was some doubt about whether or not his exposure elicited specific (c-os, c-jun) oncogenes (Phillips et al 1994).

The results of a study by Prausnitz and Susskind (1962) at 9.27 GHz pulsed radiation (2 μs pulses at 500 pps) average power density 1 kW/m^2, contribute very little to the debate. Swiss mice were exposed for 4.5 min per day for 5 days per week for 59 weeks. Rectal temperatures rose by an average of 3.3°C in the exposed group which initially comprised 200 mice, while the control group comprised only 100 mice. The study was abbreviated by an outbreak of pneumonitis and terramycin was administered during the last 3 months of irradiation. A total of 132 mice were sacrificed 7, 17 or 19 months after the initial exposure. 168 mice died, (68 without adequate post-mortem analysis). Monocytic or lymphatic "leucosis" (defined as a non-circulating neoplasm of white blood cells) and lymphatic or myeloid leukaemia (defined as a circulating "leucosis") occurred in 35% of the 60 exposed mice compared with 10% of the 40 control mice. Interpretation of the results is difficult and the study has been extensively criticised (NRPB 1991) on the grounds that "leucosis" and "leukaemia" were inadequately defined and identified, that the infection may have confounded the results, that a large proportion of mice (23%) died without a cause of death being identified and that statistical analysis was absent. An analysis (chi-square contingency table) carried out on the two groups with elevated levels of leucosis found the results to be of marginal significance ($0.1 > p > 0.05$).

A study (Spalding et al 1971) that examined the effect of 800 MHz radiation on longevity in 24 RFM mice produced inconclusive results. The mice were exposed in a highly-non-uniform-field, where the average SAR was less than 1.5 W/kg, and the peak whole-body SAR in the centre of the waveguide was sufficient to result in the death of 4 exposed mice. The authors reported a non-significant increase in the life-span of the remaining 19 exposed mice (664 days) compared to the 24 controls (645 days).

In a study using 50 AKR/J male mice, which spontaneously develop a high incidence of lymphatic leukaemia between 26 and 56 weeks of age, Skidmore and Baum (1974) irradiated with very short (5 ns rise time; 550 ns decay time) pulses of 447 kV/m peak electric field strength and pulsed at 5 Hz for 5 days per week for 33 weeks. The fraction of mice which had leukaemia at the end of exposure was 9/42 (21%) in the exposed group, compared with 11/24 (46%) in the sham-exposed group. However, the absence of a complete analysis of leukaemia